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Pollen and fungal spore sampling and analysis

Statistical evaluations

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Statistical evaluations of samples obtained from a Burkard seven-day recording volumetric pollen/spore trap were performed to determine the precision of the sampling and analysis procedures. The reproducibility of co-located traps was also investigated. The results showed that pollen grain transect counting was not significantly different, while fungal spore counting produced statistically different results. There was no statistical difference in the number of pollen and fungal spores counted between the co-located samplers. Reasons for the differences in the fungal spore counts are presented.

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To assess the impact of aerobiological particulates on the overall particulate load in southern Allegheny County in Pennsylvania, a Burkard seven-day recording volumetric pollen/spore trap was employed at the United States Department of Energy's National Energy Technology Laboratory (NETL) in South Park Township near Pittsburgh, PA.(40.3° N;79.9°W) at an elevation of 325.5 m above mean sea level. This project is part of an ambient air sampling program that was implemented in 1999 to monitor the kind and amount of fine particulate matter having an aerodynamic equivalent diameter (AED) of 2.5 microns and smaller ($PM_{2.5}$). The station was established in an attempt to address the scientific and technical issues raised by the recently passed $PM_{2.5}$ National Ambient Air Quality Standards. Although pollen and most fungal spores are larger than the 2.5 microns AED, their health implications are considered relevant to air quality, and monitoring is necessary to provide adequate data for research.

This manuscript describes methods used to estimate the precision of the sampling and analysis steps used for pollen and fungal spore counts. There have been a number of investigations (e. g. Solomon et al. 1980, Käpylä & Penttinen 1981, Pedersen & Moseholm 1993, Comtois et al. 1999) that have addressed the accuracy and precision of a variety of pollen and spore sampling devices. Although the counting methods employed in these studies were different, reproducibility among the various sampling traps was generally consistent. The current study represents a limited estimation of the precision of pollen and spore sampling and analysis. The reproducibility of two co-located Burkard seven-day recording volumetric pollen/spore traps and the associated analytical procedures were also investigated in this study. This manuscript also describes the results of a statistical analysis of the longitudinal transect method used to count pollen and fungal spores. Molina et al. (1996) found that deposition of pollen was not uniform over the collection tape. We also attempted to verify that observation.

MATERIAL AND METHODS

Experimental design

The Burkard sampler is an air-suction device (10 liters per minute) capable of gathering continuous samples during the entire pollen and fungal spore production season, usually late March through mid-November in the northeastern United States. A seven-day sampling head was used with an orifice of 2.0×14 mm. The two samplers were located 10 m above the ground surface separated by a distance of 1m. There were no obstacles to wind flow within 1m separation of the Burkard samplers. The floral assemblage for the location consisted of a secondary growth of mixed hardwood deciduous forest. The general area represents a rural to suburban transition environment.

It was assumed that the proximity of the two samplers would control for potential variability in prevailing wind speed and direction such that the collection of pollen and spores within the traps over the duration of the experiment would be negligible. The wind vanes on each Burkard sampler were observed to turn in unison each time the wind direction changed lending support to this assumption. Wind speeed averaged 11.2 kms. The average wind direction was East Northeast during the duration of the experiement.

The rotating drum inside the head was prepared with sample collecting Melenex tape coated with Lubriseal and rotated at a rate of 2 mm per hour in a clockwise direction. Thus, a 24-hour period is represented by 48 mm of sampling tape. The drum and tape were removed after a seven-day period and replaced with another drum prepared for the next sampling period. Microscope slides were then prepared for counting the number of pollen and spores trapped on the tape. A Leitz Orthoplan microscope was used with transmitted light. A $50\times$ objective lens, giving a microscopic field diameter of approximately 0.460 mm was used to count pollen, and a $100\times$ oil objective, providing a microscopic field diameter of approximately 0.187 mm was used to count fungal spores.

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Sample preparation and counting procedures

The sampling tape was sectioned into seven strips measuring 14 mm × 48 mm with each section representing one 24 hour period. Normally, each of the seven days would be counted longitudinally near the center of the slide and the total counts reported as average 24 hour concentrations after correcting the counts using the Harvard School of Public Health correction equation for the two different magnifications (M. Muilenberg: pers. comm.).

To determine if adjacent longitudinal fields counted during the same 24 hour period were significantly different, two sample slides (one for pollen taken on 9-3-99 and one for fungal spores taken on 9-5-99) were selected for counting. The counting began at the center of the tape. For pollen, 104 longitudinal microscopic fields were observed and counted for the 10 transects, while 256 longitudinal microscopic fields were observed and counted for the ten transects of fungal spores. After the first counting was completed, the results were recorded and the field of view was moved up by one adjacent microscope field of view and a second counting was performed. This was repeated until a total of 10 adjacent longitudinal transects were completed for each slide. Only total number of pollen and fungal spore grains were counted. Species differentiation was not recorded.

In order to estimate the precision of the pollen grain counting, a slide taken on 9-4-99 was selected. A single transect at the center of the slide was counted six times. The mean number of pollen grains counted was 72.17. The standard deviation of the mean was 3.06 and the relative standard deviation was 4.23%. Thus, the counting component of the analytical procedure was performed with excellent precision.

RESULTS AND DISCUSSION

Summary statistics for the ten transect counts of the pollen and fungal spore slides are presented in Table I. The sum of each transect presented in the table represents the total number of pollen and fungal spores counted in each field of view along the respective ten transects.

For pollen the mean number of pollen grains observed and counted in a field of view was less than one pollen grain. The mean number of pollen grains observed per field of view in all ten transects ranged from 0.38 to 0.51. The standard deviation of the mean values was often high, indicating that the number of pollen grains in any one field of view was highly variable. The relative standard deviations of mean pollen counts in each field of view ranged from 164% to 218% among the ten transects. In some instances, pollen was not observed in some fields of view, while the adjacent field had several pollen grains. Even though the mean number of pollen grains in any one field of view was highly variable, the total number of pollen grains across any transect was much less variable as Tables I and II indicate.

In the case of the fungal spores, the mean number of fungal spores observed and counted in a field of view was always substantially greater than the number of pollen grains. The total number of fungal spores in each transect was always greater than 1000. The increased numbers of fungal spores led to improved precision of the mean number of fungal spores observed per field of view as compared with the precision of pollen grain counts. The relative standard deviation of the mean fungal spores observed per field of view ranged from 60.3% to 86.5%. Thus, the fungal spore measurements had improved relative standard deviations as compared to the pollen grains measurements.

Table I. *Transect Summary Statistics.*

A. Pollen Counts

Transect	Sum	Mean	Standard Deviation	Relative Standard Deviation %
1	44	0.42	0.78	186
2	41	0.39	0.74	190
3	53	0.51	0.87	171
4	41	0.39	0.85	218
5	46	0.44	0.86	195
6	44	0.42	0.91	217
7	39	0.38	0.75	197
8	41	0.45	0.74	164
9	46	0.44	0.74	168
10	44	0.42	0.75	179

B. Fungal Spore Counts

Transect	Sum	Mean	Standard Deviation	Relative Standard Deviation %
1	1295	5.04	4.11	81.6
2	1444	5.62	3.66	65.1
3	1261	4.91	2.96	60.3
4	1381	5.37	4.42	82.3
5	1449	5.83	3.68	63.1
6	1538	5.98	3.03	50.7
7	1597	6.21	3.89	62.6
8	1425	5.54	4.79	86.5
9	1214	4.72	2.86	60.6
10	1185	4.61	3.40	73.8

To determine if there was a statistical difference ($p \leq 0.05$) between the 10 adjacent transect counts for the respective pollen and fungal spore slides, the one-way analysis of variance model was used (Table II). For the pollen slide, a 10×104 matrix was used; for the fungal spore slide, a 10×256 matrix was used. Data from each slide followed a normal distribution so this assumption of the variance model was met. A significant difference ($p \leq 0.05$) for the pollen counts did not emerge between the transects. However, the fungal spore counts were significantly different ($p \leq 0.05$) between the ten transects. Reasons for the statistical difference between the fungal spore counts (Table II) may be due to the fact that fungal spores are smaller than pollen grains and a smaller area of the slide is usually examined for fungal spores. Fungal spores are normally more concentrated in air than pollen (Sterling et al. 1999). The pollen counts taken at different longitudinal transects on the same slide in this study did not prove to be significantly different. Molina et al. (1996) report in their study of pollen grain counts that four longitudinal transects on the same slide were not equivalent. A difference between the current investigation and Molina et al. (1996) is that 10 longitudinal transects were observed in this study while four were used by Molina et al. (1996). Molina et al. (1996) proposed that there is a loss of pollen grains from the center of the tape towards the edges and that some of the pollen grains were deposited outside the 14 mm tape width. This might also occur with fungal spores and would account for the differences in our 10 transect counts. Alternately, particle charging could conceivably

Table II. *One Way Analysis of Variance.*

A. Pollen Counts

	Sum of Squares	df	Mean Square	F	Significance
Between Groups	1.33	9	0.15	0.23	0.99
Within Groups	661.26	1030	0.64		
Total	662.59	1039			

B. Fungal Spore Counts

	Sum of Squares	df	Mean Square	F	Significance
Between Groups	697.9	9	77.45	5.57	0.00
Within Groups	35615.3	2560	13.19		
Total	36312.4	2569			

result in nonuniform traverse deposition of particles. Other factors such as counter errors could also contribute to this problem; for example, some colorless fungal spores are difficult to see, even by a highly trained microscopist. The counter may not quantitatively observe all colorless fungal spores. Often, fungal spores tend to clump together as shown in Fig. 1B. This may create difficulty in observing all individual fungal spores. Thus, the current study represents a limited estimation of pollen and spore sampling and analysis from one slide taken over a 24 hour period when the wind speed varied between less than 1km/hr. to about 27 km/hr.

The sampling reproducibility of two co-located Burkard seven-day recording volumetric pollen/spore traps and the associated analytical procedures were also investigated. The number of pollen and fungal spores sampled and observed each day from each sampler is given in Table III). The two tailed t-test of significance was used to test differences between the samples (Table IV). The two tailed t-test uses the pooled estimate of the variation between the data groups, i.e. sampler A and sampler B with the significance determined from the difference between the means and standard deviations of the data groups. As presented in Table IV, the t-value relative to the degrees of freedom (df) shows a higher value than the $p \leq 0.05$ significance level of acceptance. For these analyses, pollen and fungal spore data collected over a 14 day sampling period from 4-18-00 to 5-01-00 were counted along one longitudinal transect near the center of each slide.

Table III. *Number of pollen and fungal spores observed by two co-located Burkard seven-day recording volumetric pollen/spore traps.*

	SAMPLER A		SAMPLER B	
Day	Number of pollen grains	Number of spores	Number of pollen grains	Number of spores
1	29	195	30	175
2	71	142	62	160
3	490	143	363	132
4	49	263	107	260
5	17	161	19	181
6	151	202	184	158
7	447	332	458	362
8	344	194	312	191
9	119	387	171	415
10	193	955	180	941
11	161	414	206	249
12	632	113	767	125
13	432	341	358	424
14	265	255	171	259

For both the pollen and fungal spore counts a significant difference ($p-0.05$) did not emerge between the samplers (Table IV). This is consistent with the investigation of Pedersen and Moseholm (1993) who also studied two

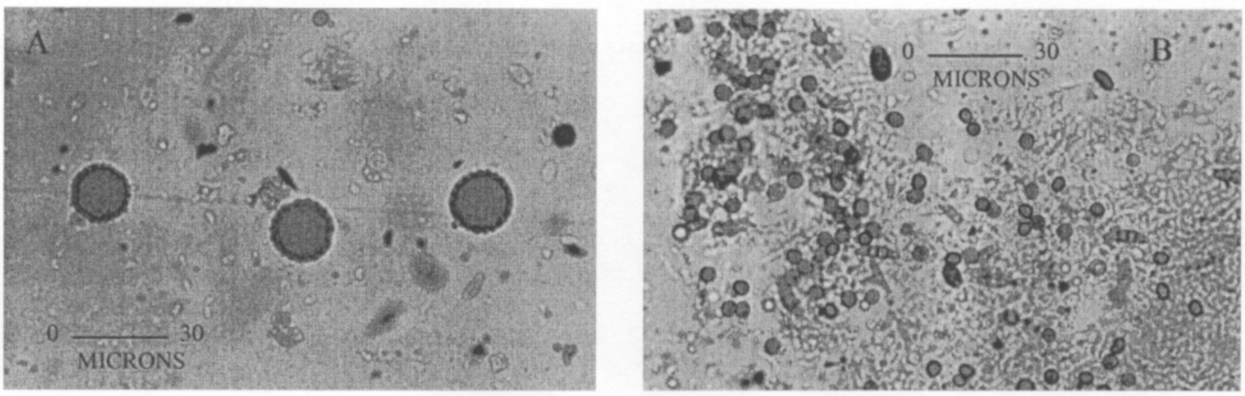

Fig. 1. LM micrographs of (A) Pollen grains and (B) Fungal spore grains sampled at NETL Pittsburgh PA.

Table IV. *Two tailed t-Test.*

Pollen Counts

	Paired Differences					
	Mean	Std. Deviation	Std. Error Mean	t	df	Significance
Sampler A Sampler B	0.86	67.50	18.04	0.48	13	0.96

Fungal Spore Counts

	Paired Differences					
	Mean	Std. Deviation	Std. Error Mean	t	df	Significance
Sampler A Sampler B	4.64	54.81	14.65	0.32	13	0.76

co-located Burkard samplers. They found that the variation between samplers is negligible.

The correlation coefficients between the data from co-located samplers were 0.94 for the pollen data and 0.97 for the fungal spore data. These high correlations for both the pollen and spores collected and analyzed over the fourteen day period demonstrate that trends in the data for the respective samplers were excellent.

CONCLUSIONS

The precision associated with counting the number of pollen grains in a transect was excellent. When a single transect near the middle of a slide was selected and pollen grains counted six times, the mean number of pollen grains counted was 72.17. The standard deviation of the mean was 3.06 and the relative standard deviation was 4.23%. The number of pollen grains sampled and counted in ten adjacent transects of the same slide were not statistically different. Conversely, the number of fungal spores sampled and counted in ten adjacent longitudinal transects were statistically different. The instrument-to-instrument repeatability of two co-located Burkard seven-day recording volumetric pollen/spore traps was studied. No statistical difference in the number of pollen or fungal spores was observed for the two co-located instruments. The day-to-day trends in the number of pollen and fungal spores sampled, observed and counted on one instrument were closely followed by the co-located instrument.

The sampling and analysis of aerobiological grains has some minor inherent problems associated with precision and identification. Various methods are used to minimize these problems, although not all of the problems have been solved. This paper has attempted to estimate the precision of the sampling and analysis of pollen and fungal spores obtained using the Burkard seven-day recording volumetric pollen/spore trap, and to estimate the reproducibility obtained when using co-located samplers. Lastly, some difficulties associated with observing and counting individual grains are delineated and possible causes are provided.

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REFERENCES

Comtois, P., Alcazar, P. & Neron, D. 1999. Pollen count statistics and its relevance to precision. – Aerobiologia 15: 19–28.

Käpylä, M. & Penttinen, A., 1981. An evaluation of the microscopic counting methods of the tape in Hirst-Burkard pollen and spore trap. – Grana 20: 131–141.

Molina, R.T., Rodriquez, A.M.& Palacios, I.S., 1996. Sampling in aerobiology. Differences between traverses along the length of the slide in Hirst spore traps. – Aerobiologia 12: 161–166.

Pedersen, B. V. & Moseholm L. 1993. Precision of the daily pollen count. Identifying sources and variation using variance component models. – Aerobiologia 9: 15–26.

Solomon, W. R., Burge, H. A., J.R. Boise, & Becker, M. 1980. Comparative particle recoveries by the retracting rotorod, rotoslide, and Burkard spore trap sampling in a compact array. – Int. J. Biometeor. 24: 107–116.

Sterling, M., Rogers, C. & Levetin, E., 1999. An evaluation of two methods used for microscopic analysis of airborne fungal spore particles from the Burkard spore trap. – Aerobiologia 15: 9–18.